

# Improved synthetic method and conformation studies of polymers and copolymers of L- $\beta$ -3,4-dihydroxyphenyl- $\alpha$ -alanine with L-glutamic acid

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Eight different copolymers of L- $\beta$ -3,4-dihydroxyphenyl- $\alpha$ -alanine (L-Dopa) and L-glutamic acid with high degrees of polymerization have been synthesized by the treatment of a series of copoly(*O,O'*-dimethyl-L-Dopa,  $\gamma$ -benzyl-L-glutamate) with boron tribromide in chloroform. The conformation of poly(L-Dopa) has been established to be a right-handed helix in trimethyl phosphate on the basis of the following observations. The  $[\theta]_{222}$  and  $b_0$  values of the copolymers were almost linear with composition in trimethyl phosphate. The linear relationship between the rotation properties and composition indicates that poly(L-Dopa) has the same helical sense as that of poly(L-glutamic acid) which is a right-handed  $\alpha$ -helix.

## INTRODUCTION

In the previous papers of this series<sup>1-5</sup> we have reported the synthesis and conformational studies of poly(L- $\beta$ -3,4-dihydroxyphenyl- $\alpha$ -alanine) [poly(L-Dopa)] and poly(L-Dopa) derivatives. From the total results obtained by a study of the optical rotation, nuclear magnetic resonance (n.m.r.) and infra-red (i.r.) absorption, it was suggested that the conformation of poly(L-Dopa) was most probably a right-handed helix in trimethyl phosphate (TMP), methanol or water/TMP mixed solvents below pH 10.4 and a random coil structure in dimethyl sulphoxide or aqueous solution above pH 11.

In the present paper, we report an improved synthetic method for high molecular weight poly(L-Dopa) and the conformational studies of homopolymers and a series of random sequence copolymers of L-Dopa with L-glutamic acid (L-Glu) in order to solve the helical sense of poly(L-Dopa) in solution. The conformation of the homo- and copolymers was studied by circular dichroism (c.d.) and optical rotatory dispersion (o.r.d.) measurements.

## EXPERIMENTAL

### Polypeptides

The preparations of high molecular weight poly(*O,O'*-dimethyl-L-Dopa) and copolymers of *O,O'*-dimethyl-L-Dopa with  $\gamma$ -benzyl-L-glutamate have been described in the previous paper<sup>5</sup>. Boron tribromide was purchased from Wako Pure Chemical Industries Ltd and was used without further purification.

*Poly(L-Dopa)*. Poly(*O,O'*-dimethyl-L-Dopa) (500 mg, 2.4 mmol) was dissolved in chloroform (100 ml), cooled to  $-10^\circ\text{C}$  and boron tribromide (6.0 g, 24 mmol) in chloroform (50 ml) was added dropwise with stirring<sup>6</sup>. Stirring was continued at  $-10^\circ\text{C}$  for 1 h, at  $25^\circ\text{C}$  for 6 h and finally at  $50^\circ\text{C}$

for 1 h. The reaction mixture was evaporated to a small volume under reduced pressure, triturated with ether and dried. The residue was washed thoroughly with water, centrifuged and dried: yield, 425 mg (98.5%). Calculated for  $(\text{C}_9\text{H}_9\text{O}_3\text{N})_n$ : C, 60.33; H, 5.06; N, 7.82%. Found: C, 60.53; H, 5.07; N, 7.94%. The polypeptide showed i.r. absorptions at 3275, 2950, 2850, 1665 and  $1548\text{ cm}^{-1}$  and the ether band of the starting polypeptide at  $1030\text{ cm}^{-1}$  disappeared.

*Poly(L-Glu)*. Poly( $\gamma$ -benzyl-L-glutamate) (500 mg, 2.3 mmol) was treated as above except that five equivalent amounts of boron tribromide (2.9 g, 11.4 mmol) were used; yield, 290 mg (98.0%). Calculated for  $(\text{C}_5\text{H}_7\text{O}_3\text{N})_n$ : C, 46.51; H, 5.47; N, 10.85%. Found: C, 46.90; H, 5.47; N, 10.63%. The ester band of the starting polypeptide at  $1732\text{ cm}^{-1}$  disappeared.

*Copolymers of L-Dopa with L-Glu*. Copolymers of *O,O'*-dimethyl-L-Dopa with  $\gamma$ -benzyl-L-glutamate were also treated as above. The unprotected copolymers obtained are listed in Table I.

### Methods

Ultra-violet (u.v.), i.r., c.d. and o.r.d. spectra were measured on UVIDEC-1, IR DS-301, CD J-40A and ORD/UV 5 instruments, respectively, all made by the Japan Spectroscopic Co. Ltd. For rotation measurements, cells with path lengths of 1–10 mm were used under nitrogen flush. The concentrations of the sample were in the 0.01–0.03% (c.d.) and 0.67–0.90% (o.r.d.) range. The experimental data were expressed in terms of mean residue ellipticity  $[\theta]$  (degree  $\text{cm}^2/\text{dmol}$ ) for c.d. or molar extinction coefficient  $\epsilon$  for u.v. The parameter,  $b_0$ , derived from the Moffitt–Yang equation was calculated from o.r.d. curves using  $\lambda_0 = 212\text{ nm}^7$ .

Table 1 Yield of copoly(L-Dopa, L-Glu)

Starting copolymer		Product L-Dopa content <sup>b</sup>		Yield <sup>c</sup>	
Composition <sup>a</sup>	DP	BBr <sub>3</sub> (g)	(mol %)	mg	%
MeDopa <sup>100</sup>	320	6.04	100	425.3	98.5
MeDopa <sup>95</sup> BzGlu <sup>5</sup>	130	5.89	96.2	418.0	98.2
MeDopa <sup>90</sup> BzGlu <sup>10</sup>	120	5.71	91.4	415.3	99.4
MeDopa <sup>80</sup> BzGlu <sup>20</sup>	80	5.39	78.4	391.0	96.7
MeDopa <sup>67</sup> BzGlu <sup>33</sup>	80	5.00	70.2	376.6	97.7
MeDopa <sup>50</sup> BzGlu <sup>50</sup>	110	4.41	52.2	355.0	98.4
MeDopa <sup>25</sup> BzGlu <sup>75</sup>	130	3.63	28.9	317.4	97.0
BzGlu <sup>100</sup>	470	2.86	0.0	290.0	98.0

<sup>a</sup> Mol % of the starting *N*-carboxyanhydrides to prepare the protected copolymers; MeDopa = *O,O'*-dimethyl-L-Dopa and BzGlu =  $\gamma$ -benzyl-L-glutamate; <sup>b</sup> determined by the u.v. absorbance at 283 nm; <sup>c</sup> 500 mg of the starting copolymers were used

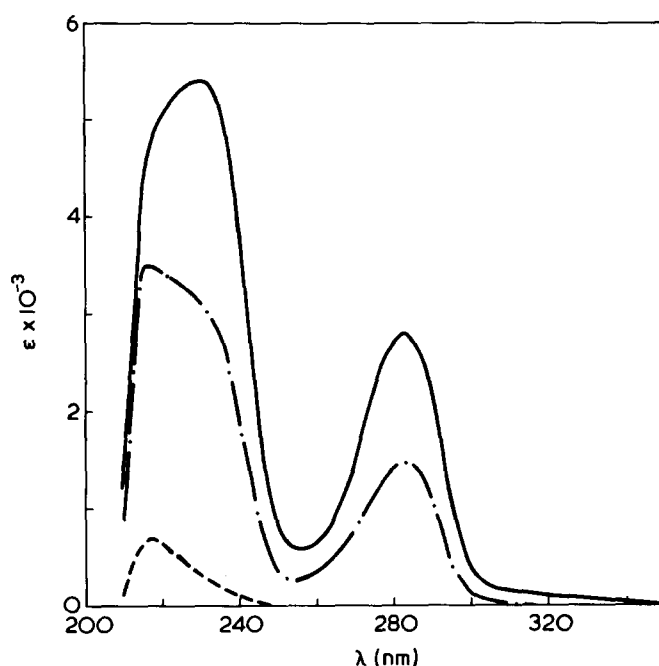


Figure 1 U.v. spectra of homo- and copolymers in TMP at 25°C: —, poly(L-Dopa); - · - · -, copoly(L-Dopa<sup>52</sup>, L-Glu<sup>48</sup>); - - - -, poly(L-Glu)

## RESULTS AND DISCUSSION

### Synthesis

Boron halides have been used for the cleavage of methyl ethers<sup>8,9</sup>, benzhydryl<sup>10</sup> and benzyl esters<sup>6</sup> and tert-butyloxycarbonyl<sup>11,12</sup> and carbobenzoxy amine protecting groups<sup>13</sup>. A recent report that the peptide bond was completely unchanged and the products were not racemized (optical purity > 99.9%) by boron tribromide<sup>6</sup> prompts us to synthesize high molecular weight poly(L-Dopa) using *O,O'*-dimethyl protecting groups. In our previous paper we have reported the synthesis of poly(L-Dopa) via poly(*O,O'*-dicarbobenzoxy-L-Dopa)<sup>3</sup>. The molecular weight of the resulting poly(L-Dopa), however, was as high as 11 000 [degree of polymerization (*DP*) = 60] since it is difficult to prepare the starting materials, pure *N,O,O'*-tricarbobenzoxy-L-Dopa and pure *O,O'*-dicarbobenzoxy-L-Dopa *N*-carboxyanhydride, as monomers. Although there was no problem in discussing the conformation of the polypeptide with *DP* = 60 we required the polypeptide to have a value of *DP* at least > 100. *O,O'*-dimethyl-L-Dopa can easily be synthesized and converted to its pure *N*-carboxyanhydride

by phosgene. The resulting poly(*O,O'*-dimethyl-L-Dopa) had an estimated molecular weight of 67 000 (*DP* = 320) and was converted easily to poly(L-Dopa) by boron tribromide. Thus, the synthetic route to high molecular weight poly(L-Dopa) and its copolymers has been established.

### Conformation

*U.v. spectra.* The u.v. absorption spectra of poly(L-Dopa) and poly(L-Glu) in TMP are shown in Figure 1. The  $\epsilon$  values for poly(L-Dopa) were  $\epsilon_{283} = 2800$  and  $\epsilon_{230} = 5400$  in TMP. Poly(L-Glu) showed a small absorption peak at 217.5 nm with  $\epsilon_{217.5} = 700$  in TMP, and the spectrum differs considerably from the spectrum in aqueous solution<sup>14</sup>. The u.v. spectra of copolymers lay between the two spectra and the u.v. spectrum of copoly(L-Dopa<sup>52</sup> L-Glu<sup>48</sup>) as an example is also shown in Figure 1.

*C.d. spectra.* The c.d. spectra of poly(L-Dopa), copoly(L-Dopa, L-Glu) and poly(L-Glu) in TMP are shown in Figure 2. In TMP, poly(L-Dopa) showed three dichroic bands at 285, 237 and 204 nm with  $[\theta]_{285} = 2300$ ,  $[\theta]_{237} = 3200$  and  $[\theta]_{204} = 66\,000$ , respectively. The positions and magnitudes of the three dichroic bands of poly(L-Dopa) changed with increasing L-glutamic acid content, that is, a band with  $[\theta]_{285} = 2300$  disappeared, a band with  $[\theta]_{237} = 3200$  changed sign and increased in magnitude to  $-33\,000$  and a strong band with  $[\theta]_{204} = 66\,000$  decreased and changed in magnitude to  $[\theta]_{207} = -33\,000$ .

Figure 3 shows the dependence of  $[\theta]_{222}$  on the composition of copoly(L-Dopa, L-Glu). The  $[\theta]_{222}$  ellipticity values of the copolymers in TMP were almost linear. The linear relationship between the  $[\theta]_{222}$  and composition indicates that poly(L-Dopa) has the same helical sense as that of poly(L-Glu) which exists as a right-handed  $\alpha$ -helix<sup>15</sup>.

*O.r.d. spectra.* The o.r.d. curves of this series of copolymers were measured in TMP over the wavelength range 320–600 nm. The  $b_0$  value for poly(L-Dopa) in TMP was found to be 350. The  $b_0$  value for helical poly(L-Glu) was found to be  $-530$ .  $b_0$  values varied linearly from 350 to  $-530$  for this series of copolymers. This again indicates that the same helical sense is maintained in these copolymers, i.e. a right-handed helix.

Previously, we have reported that both o.r.d. and c.d. spectra of poly(L-Dopa) are anomalous and give little information about its conformation<sup>3</sup>. From the i.r. frequencies for the amide I band and the n.m.r.  $\alpha$ -CH chemical shifts, it was estimated that the helical sense was most probably right-

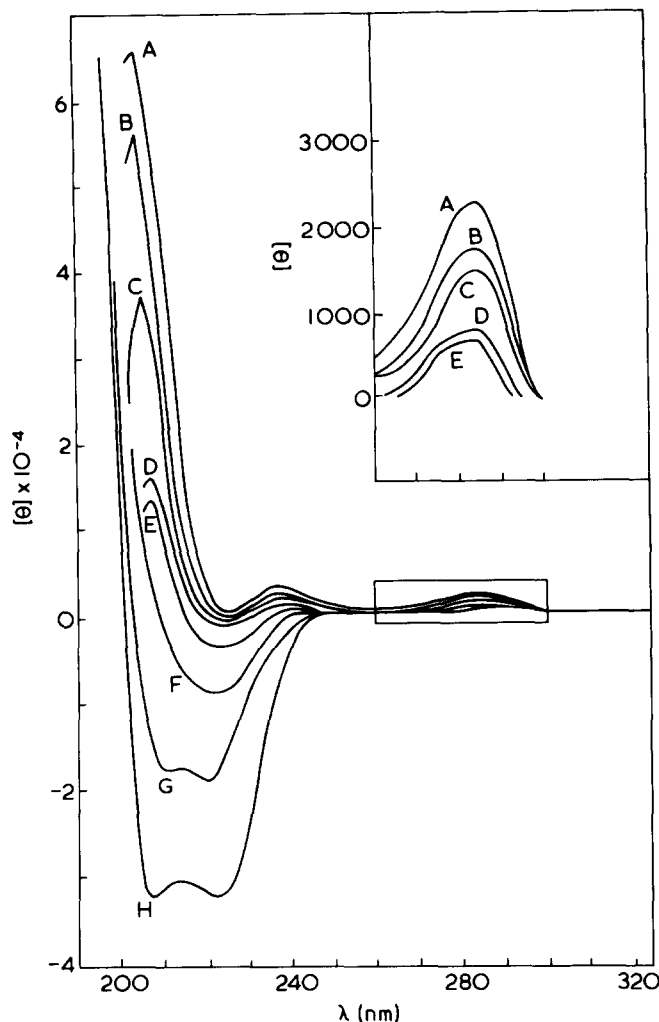


Figure 2 C. d. spectra of copoly(L-Dopa, L-Glu) in TMP at 25°C. Mol % of L-glutamic acid in copolymers: A, 0%; B, 4%; C, 9%; D, 22%; E, 30%; F, 48%; G, 71%; H, 100%

handed. Since the  $[\theta]_{222}$  and  $b_0$  values varied linearly with the composition of copoly(L-Dopa, L-Glu) as has been found for poly( $\beta$ -benzyl-L-aspartate)<sup>16,17</sup>, poly(*O*-carbobenzoxy-L-tyrosine)<sup>18</sup>, poly(L-tyrosine)<sup>19,20</sup>, poly(L-tryptophan)<sup>21,22</sup>, poly(*O,O'*-dicarbobenzoxy-L-Dopa)<sup>4</sup> and poly(*O,O'*-dimethyl-L-Dopa)<sup>5</sup>, the copolymer study reported here gave the additional evidence that leads to the conclusion that poly(L-Dopa) is a right-handed helix. Thus, the synthetic route to poly(L-Dopa) and its copolymers is established and the helical sense of poly(L-Dopa) is confirmed to be right-handed.

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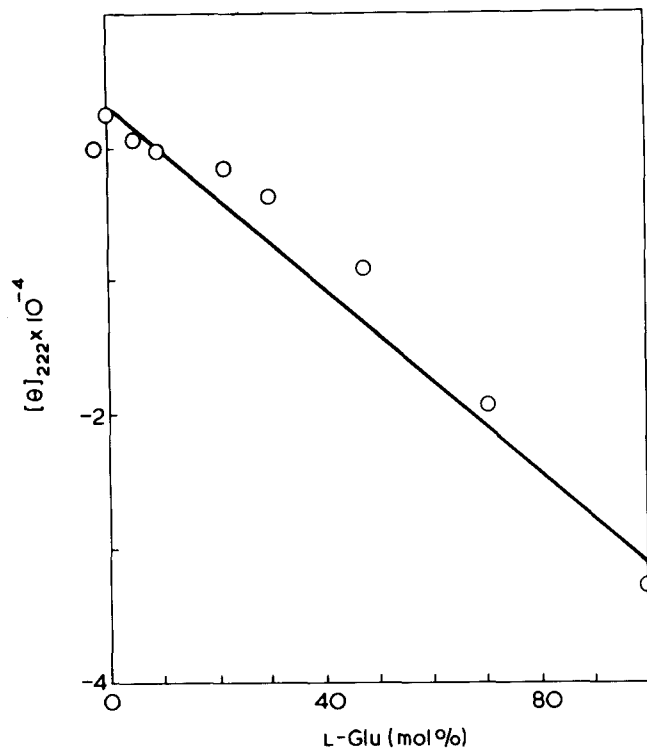


Figure 3 Dependence of  $[\theta]_{222}$  on the composition of copoly(L-Dopa, L-Glu) in TMP at 25°C

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